PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 35/12

A1

(11) International Publication Number: **WO 99/51247**

(43) International Publication Date: 14 October 1999 (14.10.99)

(21) International Application Number: PCT/US99/05349

(22) International Filing Date: 12 March 1999 (12.03.99)

(30) Priority Data:
60/080,533 3 April 1998 (03.04.98) US

(71) Applicant: OSIRIS THERAPEUTICS, INC. [US/US]; 2001 Aliceanna Street, Baltimore, MD 21231-2001 (US).

(72) Inventors: MOSCA, Joseph, D.; 4201 Blue Barrow Ride, Ellicott City, MD 21042 (US). McINTOSH, Kevin, R.; 4225 Blue Barrow Ride, Ellicott City, MD 21042 (US).

(74) Agents: SQUIRE, William et al.; Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein, 6 Becker Farm Road, Roseland, NJ 07068 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: USE OF HUMAN MESENCHYMAL STEM CELLS TO INDUCE T-CELL APOPTOSIS

(57) Abstract

The present invention provides a method for inducing antigen-specific T-cell lymphocyte elimination using human mesenchymal stem cells as antigen presenting cells which additionally express a molecule that induces T-cell apoptosis.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

USE OF HUMAN MESENCHYMAL STEM CELLS TO INDUCE T-CELL APOPTOSIS

This application is based on and claims priority of U.S. provisional application serial no. 60/080,533 filed April 3, 1998.

The present invention relates to the field of inducing death of specific T-lymphocyte cells which are deleterious to an organism. The present invention relates particularly to the area of autoimmune disease in humans.

Background of the Invention

The function of the immune system is to eliminate foreign cells which may contain pathogens, while maintaining unresponsiveness or "tolerance" against self-antigens. In a normal immune response, activation of naive T-cells requires recognition of a foreign antigenic fragment bound to a self MHC molecule and the simultaneous delivery of a co-stimulatory signal by a specialized antigen-presenting cell. T cell tolerance is achieved 1) in the thymus where thymocytes reactive for self-peptides are eliminated by clonal deletion (central tolerance), and 2) in the periphery by exposure to self-antigens under tolerogenic conditions (peripheral tolerance). Peripheral tolerance is manifested by clonal anergy, and by clonal deletion where autoreactive cells are eliminated.

Clonal deletion can also result from expression of cell death molecules on the antigen presenting cells. Classic examples of death molecules are Fas ligand (FasL) and TRAIL ligand, which ligate their receptors, Fas and DR4, respectively,

on activated T cells, inducing apoptosis of the T cells. The interaction of CD27, a member of the TNFR superfamily, and the CD27-ligand (CD70) also induces T cell apoptosis.

However, the immune system may generate a response against self-constituents, as happens in autoimmune disease. Autoimmune disease, wherein antibodies or T cells attack self proteins, may be caused by abnormal immune response. The cause may be an autoreactive T cell component, the T cells may themselves be pathogenic, or T cells may help trigger autoreactive B cells to produce antibodies to self antigens. Patients with autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease and myasthenia gravis, are either inadequately treated with existing non-selective drug therapies, or experience deleterious side effects from long-term immunosuppressive treatment.

Infusion of individuals with drugs that prevent T-cell activation can inhibit immune cell response, but these treatments result in general immune suppression, toxicity and sometimes death due to opportunistic infections. Because of the toxicity and incomplete response rate of conventional treatment of autoimmune diseases, alternative approaches are needed for patients who cannot withstand or do not respond to drug therapy.

Summary of the Invention

It has been discovered that human mesenchymal stem cells can be used to deliver antigens to the immune system for interaction with T cells. Mesenchymal stem cells can further be used to present to the immune system molecules that induce apoptotic death in cells of the immune system that express receptors for the molecules.

Accordingly, the methods of the present invention are particularly useful for eliminating, reducing or ameliorating unwanted or activated T cell responses and

can be used as a method to treat or inhibit specific unwanted or abnormal immune responses such as occurs in autoimmune disease.

In one aspect the method involves reducing, ameliorating or eliminating T cells that have been activated against an antigen by administering to a subject autologous human mesenchymal stem cells which have been modified to present such antigen, and to express a molecule that induces apoptosis of activated T cells. The mesenchymal stem cells can be used to deliver to the immune system a molecule that induces apoptosis of activated T cells since activated T cells carry a receptor for the molecule. This results in the deletion of activated T lymphocytes and in the suppression of an unwanted immune response. In accordance with an aspect of the invention, autologous human mesenchymal stem cells are modified to express a cell death molecule. In a preferred embodiment, the mesenchymal stem cells express the cell death molecule Fas ligand which will interact with the Fas receptor found on activated T cells.

Thus, the method of the present invention provides administering to a host a human mesenchymal stem cell that (i) has been modified to have at least one exogenous antigen fragment bound to a primary surface molecule of the cell such that the antigen fragment is presented to the immune system, and (ii) has been modified to express a cell death molecule. The mesenchymal stem cell presents the antigen and thereby interacts with T cells that have previously been activated. The mesenchymal stem cells of the invention further contain exogenous genetic material that codes for a molecule that induces activated T cell apoptosis. Preferably, the exogenous genetic materials are in one or more expression vectors.

In another aspect, the mesenchymal stem cells are modified to deliver to the immune system a molecule that induces activated T cell elimination. The mesenchymal stem cells, which may be allogeneic to the host, are modified to express a cell death molecule such as Fas ligand or TRAIL. When the mesenchymal stem cell comes into contact with an activated T cell, apoptosis of the activated T cell will be induced.

The mesenchymal stem cell-antigen presentation system described herein has a wide range of applications, including but not limited to, deletion of large numbers of antigen-specific T cells for use in immunotherapy against, *inter alia*, autoimmune disease.

Detailed Description of Preferred Embodiments

The invention relates to methods for reducing, inhibiting or eliminating an immune response to an antigen, *in vivo*, by employing human mesenchymal stem cells to present antigen and to simultaneously present "a cell death molecule", a molecule that induces immune cell apoptosis. The administration of these modified mesenchymal stem cells results in the deletion of activated T cells and thus a reduction of the T cell response. The human mesenchymal stem cells are preferably autologous to the recipient of the human mesenchymal stem cells.

Accordingly, the invention relates to a method of eliminating activated T cells by administering, *in vivo*, mesenchymal stem cells which deliver a specific antigen to T cells, and in addition are modified to express a cell death molecule. The present invention is based in part on the discovery that human mesenchymal stem cells do not provide costimulatory signals to fully stimulate T cells. Therefore, when antigen bearing mesenchymal stem cells are present in the immune system, the mesenchymal stem cells present the antigen without providing the costimulatory signal required for T cell activation.

In one embodiment of the invention, the mesenchymal stem cells are modified to present antigen to T cells by contacting the mesenchymal cells with antigen, *in vitro*, prior to contact with the T cells. For human mesenchymal stem cells modified to have at least one exogenous antigen fragment, the antigen can be a protein, a polypeptide, lipid or glycoprotein bound to a primary surface molecule of the cell. Thus, in this embodiment, the mesenchymal stem cell is contacted with at least one antigen (antigen-pulsing) which the mesenchymal stem cell processes into an antigen fragment.

The mesenchymal stem cells can alternatively be genetically manipulated to express an antigenic molecule. Thus, in another embodiment, the mesenchymal stem cell contains exogenous genetic material that codes for at least one exogenous antigenic polypeptide, which the mesenchymal stem cell expresses, processes into an antigen fragment and presents to the T cells.

In a preferred embodiment of this aspect of the invention, the mesenchymal stem cells are modified to present an autoantigen, for example, an autoantigen that mediates the immune response in an autoimmune disease. In accordance with this aspect, the mesenchymal stem cells are preferably autologous to the recipient of the mesenchymal stem cells. This method can be used to reduce or inhibit a T cell immune response involved in autoimmune disease, for example, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease and myasthenia gravis. By using mesenchymal stem cells that present the autoantigen against which the T cells have been activated, such activated T cells will recognize the presented antigen.

In accordance with this embodiment of the invention, the mesenchymal stem cells are also modified to express a molecule that will induce T cell apoptosis, i.e., a cell death molecule. As defined herein a "cell death molecule" is a molecule that interacts or binds with its cognate receptor on an activated T cell, the interaction inducing T cell death or apoptosis.

Fas mediates apoptosis of recently activated T cells which are again exposed to stimulation (Parijs, et al, 1996). Fas is a type I membrane receptor that, when crosslinked by its cognate ligand, induces apoptosis in a wide variety of cells. The interaction between the Fas molecule (CD95) on target cells and its ligand Fas L on activated T cells results in receptor aggregation, which transduces signals leading to apoptosis of the target cell. The Fas system has been shown to be involved in a number of cell functions *in vivo* including negative selection of thymocytes,

maintaining immune privilege sites within the body, and cytotoxic T-lymphocyte (CTL)-mediated cytotoxicity (Green and Ware, PNAS 1997).

Other members of the tumor necrosis factor receptor (TNFR) family have roles in programmed cell death: DR4 TRAIL receptor interacts with TRAIL ligand which can induce apoptosis in a variety of transformed cell lines (G. Pan SCIENCE, 1997); and the interaction of CD27 and its ligand CD70 (Prasad et al, PNAS 1997) also induces apoptosis. Whereas FasL expression is restricted to stimulated T cells and cites of immune privileges, TRAIL is detected in many normal tissues. Both TRAIL-ligand and CD70, but not Fas-ligand, are expressed on unmanipulated human mesenchymal stem cells. Activated, but not resting, T cells express the TRAIL receptor and CD27. Thus, in accordance with the present invention, the mesenchymal stem cells can be induced to express an endogenous cell death molecule or can be genetically engineered to express exogenous molecules that cause cell death.

It is believed that the mesenchymal stem cells which present an antigen to which T cells have been previously activated cause the T cells to be drawn to such mesenchymal stem cells. The activated T cells express either TRAIL-receptor, Fas or CD27 on the T cell. The engagement of these receptors with their ligands on the mesenchymal stem cells results in T cell death via apoptosis. Other ligands either present within the mesenchymal stem cell or introduced into the mesenchymal stem cell can bind to their cognate receptors on the activated T cells to induce apoptosis. In this manner, mesenchymal stem cells administered to an individual can delete autoreactive cells, reducing the severity or incidence of autoimmune disease.

An advantage of the method of the present invention over current treatment for autoimmune disease is specificity; mesenchymal stem cells can be targeted to reduce a specific immune response while reducing or eliminating the effect on other segments of the immune system. The elimination of an antigen specific immune response enables the treatment of or prevention of an unwanted or abnormal immune response to a specific antigen. The methods of the present invention are

particularly applicable to therapy of autoimmune disease and preferably eliminate the response to autoantigen specifically, while reducing or eliminating the effect on other aspects of the immune system.

The invention can be utilized for treatment of autoimmune diseases where the autoantigen mediating the disease is known. The method involves genetically engineering mesenchymal stem cells, to express an autoantigen in order to induce specific immunotherapy to inactivate or eliminate abnormal immune responses. Accordingly, the invention encompasses administering the mesenchymal stem cells to a host as a method for the treatment of autoimmune diseases such as myasthenia gravis or rheumatoid arthritis.

In another aspect of the invention, the mesenchymal stem cells are modified to express a molecule that will induce T cell apoptosis, without modification to present an antigen against which the T cells have been activated. The mesenchymal stem cells thus modified will have a nonspecific effect on the immune system, i.e., will eliminate activated T cells at or near the site of administration of the mesenchymal stem cells. Upon contact with the mesenchymal stem cells, apoptosis of the activated T cells will be induced.

Mesenchymal stem cells are the formative pluripotential blast cells found, *inter alia*, in bone marrow, blood, dermis and periosteum. These cells can be expanded in culture, for example by methods described for isolating, purifying, and greatly replicating these cells in culture, *i.e., in vitro,* in Caplan and Haynesworth, U.S. Patent No. 5,486,359.

The human mesenchymal stem cells of the invention can be engineered (transduced or transformed or transfected) with genetic material of interest. The engineered human mesenchymal stem cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying exogenous genes therein. The culture conditions, such as temperature,

pH and the like, can be those previously used with engineered human mesenchymal stem cells. See, for example, Gerson *et al.*, U.S. Patent No. 5,591,625.

Unless otherwise stated, genetic manipulations are performed as described in Sambrook *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).

The mesenchymal stem cells and method of the invention can be appropriately applied to treatment strategies requiring immunosuppressive reagents. Accordingly, the present invention provides for the modification of and expansion of mesenchymal stem cells *in vitro* for use in cellular immunotherapy, and the *in vivo* administration of the immunosuppressive mesenchymal stem cells for treating or ameliorating unwanted immune responses. One aspect of the invention is the development of the mesenchymal stem cells into a vehicle for presenting antigen and delivering a cell death molecule for eliminating a specific cellular response.

The dosage of the active ingredient varies within wide limits and will, of course be fitted to the individual requirements in each particular case. In general, in the case of parenteral administration, it is customary to administer from about 0.5 to about 5 million cells per kilogram of recipient body weight. The number of cells used will depend on the weight and condition of the recipient and other variables known to those of skill in the art. The cells can be administered by a route which is suitable for the particular disease state to be treated. The antigen-modified mesenchymal stem cells can be targeted to a particular tissue or organ such as bone marrow.

The cells can be suspended in an appropriate diluent, at a concentration of from about 5×10^6 to about 50×10^6 cells/ ml. Suitable excipients for injection solutions are those that are biologically and physiologically compatible with the recipient, such as buffered saline solution. The composition for administration should be sterile, stable and physiological acceptable.

It is contemplated that the mesenchymal stem cells of the present invention can be used in conjunction with current modes of treating autoimmune disease. By ameliorating the severity of the immune response in autoimmune disease, the amount of drug used in treatment and/or the frequency of administration of drug therapy can be reduced, resulting in alleviation of general immune suppression and unwanted side effects.

What Is Claimed Is:

1. A method of eliminating T cells comprising administering to a host a human mesenchymal stem cell which expresses a cell death molecule.

2. The method of claim 1 wherein the cell death molecule is selected from the group consisting of Fas Ligand, TRAIL ligand and CD27 ligand.

3. A method of reducing T cells activated against an antigen, comprising administering to a host human mesenchymal stem cells which present the antigen against which the T cells have been activated, and which express a cell death molecule.

4. The method of claim 3 wherein the antigen is an autoantigen.

5. The method of claim 3 wherein the mesenchymal stem cells are autologous to the host.

6. The method of claim 3 wherein the cell death molecule is selected from the group consisting of Fas Ligand, TRAIL ligand and CD27 ligand.

7. Use of mesenchymal stem cells which express a cell death molecule for the preparation of a composition for eliminating T cells.

8. Use of mesenchymal stem cells which present an antigen and which express a cell death molecule for the preparation of composition for reducing T cells activated against the antigen.

INTERNATIONAL SEARCH REPORT

Inte· ·onal Application No
PCT/US 99/05349

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K35/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BRUDER S P ET AL: "Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and followin cryopreservation." JOURNAL OF CELLULAR BIOCHEMISTRY, (1997 FEB) 64 (2) 278-94. JOURNAL CODE: HNF. ISSN: 0730-2312., XP002109558 United States see the whole document ---	1-8
A	WO 95 35321 A (GSF FORSCHUNGSZENTRUM UMWELT ;THIERFELDER STEFAN (DE)) 28 December 1995 see the whole document ---	1-8
A	WO 94 03202 A (US HEALTH) 17 February 1994 see the whole document -----	1-8

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 1999	13.08.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mennessier, T

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/05349

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-6 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the human mesenchymal stem cells.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intr ional Application No
PCT/US 99/05349

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9535321 A	28-12-1995	DE 4421391 C	30-11-1995
		AT 175215 T	15-01-1999
		CA 2193092 A	28-12-1995
		DE 59504707 D	11-02-1999
		EP 0802924 A	29-10-1997
		ES 2127531 T	16-04-1999
		JP 10505325 T	26-05-1998
		US 5830473 A	03-11-1998
WO 9403202 A	17-02-1994	AT 151989 T	15-05-1997
		AU 688736 B	19-03-1998
		AU 4805293 A	03-03-1994
		CA 2140878 A	17-02-1994
		DE 69310182 D	28-05-1997
		DE 69310182 T	04-12-1997
		EP 0655927 A	07-06-1995
		JP 7509724 T	26-10-1995